APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 2004, p. 2021–2027 Vol. 70, No. 4
0099-2240/04/$08.00+0 DOI: 10.1128/AEM.70.4.2021–2027.2004

Size and UV Germicidal Irradiation Susceptibility of *Serratia marcescens* when Aerosolized from Different Suspending Media

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Received 21 July 2003/Accepted 20 December 2003

Experimental systems have been built in laboratories worldwide to investigate the influence of various environmental parameters on the efficacy of UV germicidal irradiation (UVGI) for deactivating airborne microorganisms. It is generally recognized that data from different laboratories might vary significantly due to differences in systems and experimental conditions. In this study we looked at the effect of the composition of the suspending medium on the size and UVGI susceptibility of *Serratia marcescens* in an experimental system built in our laboratory. *S. marcescens* was suspended in (i) distilled water, (ii) phosphate buffer, (iii) 10% fetal calf serum, (iv) phosphate-buffered saline (saline, 0.8% sodium chloride), and (v) synthetic saliva (phosphate-buffered saline with 10% fetal calf serum). At low humidity (36%), *S. marcescens* suspended in water-only medium was the most susceptible to UVGI, followed by those in serum-only medium. The count median diameters (CMDs) for culturable particles from water-only and serum-only media were 0.88 and 0.95 μm, respectively, with the measurements based on their aerodynamic behavior. The bacteria suspended in phosphate buffer, synthetic saliva, and phosphate-buffered saline had similar UVGI susceptibility and CMD at 1.0, 1.4, and 1.5 μm, respectively. At high humidity (68%) the CMD of the particles increased by 6 to 16%, and at the same time UVGI susceptibility decreased, with the magnitude of decrease related to the type of suspending medium. In conclusion, the choice of suspending medium influenced both size and UVGI susceptibility of *S. marcescens*. These data are valuable for making comparisons and deciding on the use of an appropriate medium for various applications.

Deactivation of airborne microorganisms is of interest because of the increasing incidence of new deadly infectious diseases and the increasing number of antimicrobial-resistant microorganisms (2, 20). UV germicidal irradiation (UVGI) at 254 nm has been used for deactivating airborne microorganisms for many years, especially in laboratory and medical facilities where a high standard of air quality is required (1, 8). In recent decades, research and application of UVGI in a range of environments has been implemented, such as installing upper-room UVGI in homeless shelters and schools and using UVGI systems in ventilation ducts in office buildings (1, 8, 17). Some of the major challenges of using UVGI in these facilities are the highly variable and uncontrolled environments and the complexity of microbial aerosols (10, 17). There is no recent study to confirm the success of UVGI systems in controlling the transmission of airborne disease in these applications.

Experimental UVGI exposure systems consisting of three basic components (aerosol generation unit, UVGI exposure unit, and bioaerosol sampling unit) have been built in laboratories worldwide to investigate the influence of various environmental parameters on UVGI efficacy (7, 11, 13). This information is important to explain and assess UVGI efficacy under various conditions, as well as to improve UVGI features to maximize the deactivation rate. Although experiments have been undertaken under controlled conditions, it is generally recognized that data from different laboratories might vary significantly due to differences in UVGI exposure systems and experimental conditions (1, 19).

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The composition of the suspending medium released along with the microorganisms from various sources plays a crucial role in controlling the viability, deposition site, and infectivity of the agents in respiratory systems and in determining the success of UVGI deactivation as an intervention (2, 4, 14, 17). However, few studies have focused on the influence of the suspending medium on the susceptibility of airborne agents to UVGI (15, 17, 18). In this study, we aimed to (1) compare the size and UVGI susceptibility of airborne *Serratia marcescens* in several suspending media that are commonly used in UVGI laboratory experiments (2) and to relate changes in susceptibility to size and suspending medium at two relative humidity (RH) levels in an experimental exposure system built in our laboratory. *S. marcescens*, chosen as a model bacterium in this study, has been widely used in UVGI research, having reported UVGI susceptibility values (Z) of 0.02 to 4.99 $m^2\ J^{-1}$ in air (1, 7, 8, 9). UVGI susceptibility was calculated with the following equations:

$$\frac{N_{UV}}{N_0} = e^{-ZD}$$

$$Z = \frac{\ln(N_0/N_{UV})}{D}$$

where Z (microorganism-dependent decay rate constant originated by Kethley in the 1970s, cited and used in Ko et al. [7]) equals UVGI susceptibility, measured as square meters per joule; D equals UVGI dose, a factor of light intensity and exposure time; and joules per square meter and N are the colony counts, CFU, with UV on (N_{UV}) and UV off (N_0).

MATERIALS AND METHODS

Bacterial culture. *S. marcescens* was obtained from the American Type Culture Collection (ATCC 8195) and stored at −70°C. Cells used in the experiments

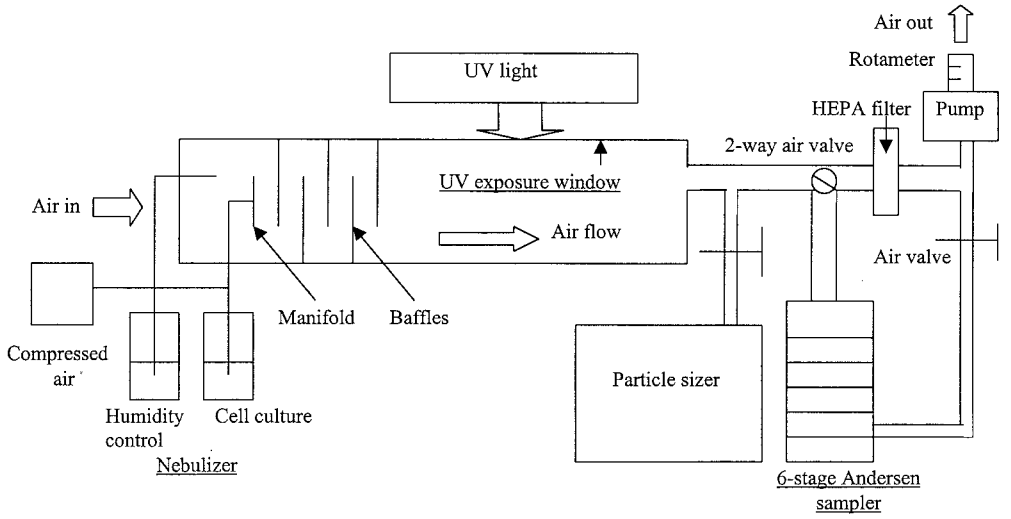

FIG. 1. Schematic diagram of the experimental UVGI exposure system.

were prepared by inoculating a loopful of 2-day-old cells grown on nutrient agar (NA) (Difco Laboratories, Detroit, Mich.) into 100 ml of nutrient broth (Difco Laboratories) and culturing the mixture at 25°C for 24 h with 200 rpm of agitation. About 1 ml of the resulting culture was pipetted into Eppendorf tubes and was washed with 1 ml of phosphate-buffered saline (PBS) (0.8% NaCl, 0.02% KH_2PO_4, 0.115% Na_2HPO_4, 0.02% KCl [pH 7.4]) twice, and harvested by centrifugation at 2,300 × *g* for 15 min. Cell aliquots were stored at −70°C for later uses. The relationship between optical density at 620 nm of the cell cultures in PBS and the colony counts was determined to be 3.95×10^{13} viable cells ml^{-1} in 1.0 U of absorbance. This value was used later as a reference for adjusting the cell densities in the experiments.

Experimental system. The details of the experimental system are shown in Fig. 1. Briefly, the system is composed of three parts: (i) aerosol generating and drying section, (ii) UVGI exposure unit, and (iii) sampler. The first part contains a six-jet Collison nebulizer (model CN-38; BGI, Waltham, Mass.) running at 20 lb/in^2. This nebulizer generated 90% of aerosols [using nonvolatile di(2-ethylhexyl)phthalate (DOP) as a reference; when volatile suspending media, e.g., water, are used, evaporation begins as soon as the aerosol is released into the air and particles become smaller than the reference size] within a size range from 0.95 to 2.2 μm, with a count median diameter (CMD) of 1.25 μm and a geometric standard deviation (GSD) of 1.29. CMD is the size at which the aerosol particles are divided into two groups; one-half of all the particles are smaller than the median size, and one-half of all the particles are larger than the median size. In this study, we measured the size of particles based on their aerodynamic behavior. The size of a particle is equal to the diameter of a spherical particle with a density of 1 g cm^{-3} and with the same settling velocity as the measured particle (6). Because many of these droplets are larger than *S. marcescens* cells (0.5 to 0.8 μm in diameter and 0.9 to 2 μm in length) (5), most droplets could contain at least one bacterial cell. However, the viscosity and salt content of the suspending medium may change the droplet size and the number of aerosolized particles compared to those for DOP. The aerosolized droplets are released into a manifold and pass through baffles to produce turbulence and introduce evenly distributed air-dried particles at the entrance into the UVGI section. When one nebulizer was used, RH was 36%, measured inside the UVGI exposure section with a HOBO H8 temperature/RH meter (Onset Computer Corporation, Bourne, Mass.). When a higher level of RH was required, another six-jet Collison nebulizer filled with distilled water was added upstream of the first nebulizer. It produced RH of 68%. The airflow through the system was maintained at 1 ft^3/min with about ±4% fluctuation, and the measured temperature was 26 ± 1°C.

The second part of the system, the chamber for UVGI exposure, is covered by a UV-transparent 279- by 254-mm fused quartz window with a height of 50.6 mm. The exposure time for the bacteria passing the window was calculated to be 7.6 s at a flow rate of 1 ft^3/min. UVGI is generated by six 36-W UV lamps (Lumalier, Memphis, Tenn.) with screens inserted beneath the UVGI fixture to control irradiance to an average level of 45 μW cm^{-2}. When passing through the chamber in 7.6 s, the dose equals 3.42 J m^{-2}. UVGI irradiance was measured with a photodetector (model SEL240, no. 3660; International Light Inc., Newburyport, Mass.). UVGI measurements were unaffected by RH. Since this UVGI dose inactivates nearly 100% of the airborne bacteria at the tested concentrations, we blocked portions of the quartz window with a 245- by 260- or a 150- by 260-mm cardboard, which reduced the UVGI doses to 0.73 and 1.84 J m^{-2}, respectively.

The third part of the chamber is designed for sampling. It contains a bypass to a high-efficiency particulate air (HEPA) filter, a one- or six-stage Andersen sampler (Andersen Samplers, Inc., Atlanta, Ga.), or a particle counter and sizer (API Aerosizer; TSI Inc., Shoreview, Minn.). Samplers were operated at 1 ft^3/min for 3 min. The whole system was set up inside a 6-ft biosafety cabinet.

Experimental procedures. The suspending media tested were (i) distilled water, (ii) phosphate buffer (PB) (0.02% KH_2PO_4, 0.115% Na_2HPO_4, 0.02% KCl [pH 7.4]), (iii) 10% fetal calf serum (Cambrex Bio Science, Walkersville, Inc., Walkersville, Md.), (iv) PBS, and (v) synthetic saliva (PBS with 10% fetal calf serum). Bacterial culture aliquots were suspended in PBS and adjusted to 0.25. This cell suspension was serially diluted 1,000-fold, and 1 ml of the final suspension was mixed with 70 ml of the suspending medium in the nebulizer. Fifty milliliters of this cell mixture was transferred into the nebulizer reservoir. The culturable cell concentrations in the nebulizer were determined before and after the run by culturing a 0.1-ml aliquot on NA with or without serial dilution. The cell densities were around 10^3 to 10^4 CFU per ml.

Before collection of the first sample, nebulization was begun and the system was equilibrated for 5 min at the UV-off setting. The airborne particles were then collected by a single-stage Andersen sampler running at 1 ft^3/min for 3 min of sampling time. For selection of the appropriate UVGI dose for the experiments, bacteria suspended in water-only medium were aerosolized continuously and exposed to a UVGI dose of either 0, 0.73, 1.84, or 3.42 J m^{-2}. Another 3-min time interval was applied to equilibrate each change in UV setting. Triplicate samples at each UV setting were collected on NA plates loaded into the one-stage Andersen sampler. The collected samples were incubated at 30°C for 2 days. The number of colonies formed on the plates was counted and converted to the corresponding corrected particle counts by using the positive-hole conversion table (Andersen Samplers, Inc.).

The UVGI dose at 0.73 J m^{-2} was selected for the experiments to investigate the effect of suspending medium on microbial UVGI susceptibility. Bacteria suspended in various media were aerosolized and exposed to UVGI at 36 or 68% RH. A six-stage Andersen biological sampler was used, and all stages were loaded with NA plates. Triplicate samples were collected under each experimental condition. The collected bacteria were incubated, the colonies were enumerated, and the number of positive holes was corrected as noted above.

Determination of the number and CMD of aerosolized particles. The numbers and particle sizes of dried droplets aerosolized from different suspending media with or without bacteria and with UV on or off were determined at 36% RH by using an API Aerosizer. The Aerosizer covering the size range 0.1 to 10 μm was operated for 1 min at 2.5 liters min^{-1} and was set for a specific gravity of 1. Note that although the Aerosizer could be set to measure particles as small as 0.1 μm, many believe that the lowest reliable measurable diameter is 0.3 to 0.5 μm (16). Duplicate samples were collected in each run. CMD and GSD for each suspend-

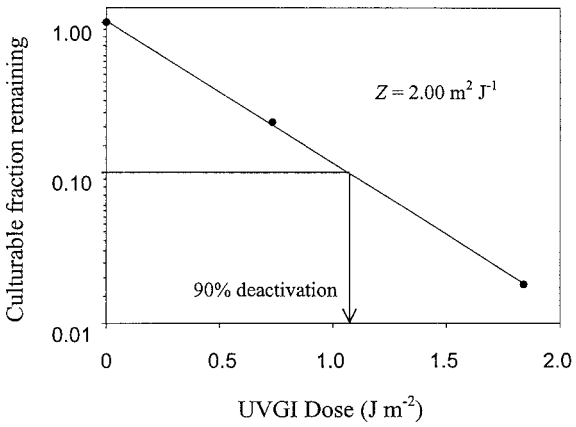

FIG. 2. Survival curve of *S. marcescens* under a range of UVGI exposures.

ing medium were determined by using log-probability plots. For culturable particles, the CMD and GSD were determined by using data from the six-stage Andersen sampler.

Statistical analysis. For each pair of exposure conditions (UV off and UV on), only the stages of the six-stage sampler that had more than 30 CFU on the NA plates at the UV-off setting were selected for calculation. This lower limit of CFU was recommended to provide sufficient statistical power for comparison purposes (11).

Confidence intervals, at $\alpha = 0.05$, were calculated to indicate the uncertainty of the replicate samplings. One-way analysis of variance at a significance level of 0.05 with Bonferroni correction for multiple comparisons (SPSS, Inc., Chicago, Ill.) was used to evaluate the statistical significance of differences in bacterial susceptibility to UV among the different suspending media at 36% RH.

RESULTS

Survival versus UVGI dose. Initial experiments conducted with a 3.42-J m^{-2} UVGI dose deactivated all bacterial cells suspended in water. Therefore, the first aim was to determine survival versus UVGI dose and the linear relationship between them on a semilog plotting paper in order to validate the choice of UVGI intensity and dose which would reflect the unique microbial-specific decay rate constant. Figure 2 shows a linear curve in a semilog scale between survival versus UVGI dose for *S. marcescens* suspended in water, which indicates that the level of UVGI intensity and dose used were above the threshold level of deactivation and self repair. The 90% deactivation dose was 1.05 J m^{-2}. Z was 2.20 ± 0.35 $m^2\ J^{-1}$. Based on these data, subsequent experiments were conducted at a UVGI dose of 0.73 J m^{-2}, at which about 22% of all cells aerosolized from water-only suspensions remained culturable after UVGI exposure. This dose made it possible to observe medium-related changes in both directions.

Determination of size distribution and CMD of culturable particles. More than 90% of culturable particles from water, PBS, and synthetic saliva were recovered on stages 4, 5, and 6 of the six-stage cascade impactor, and only these three stages were used for the PB and serum experiments. Table 1 presents particle size distributions for the culturable aerosols with different suspending media. Viable cell concentrations ranged from 4,541 to 27,227 CFU m^{-3}. This information was used to calculate CMD and GSD. The CMD increased with increasing concentrations of solutes in the suspending medium (PBS > synthetic saliva > PB > serum-only > water-only). Overall, the GSD of culturable particles was around 1.56, except for the particles aerosolized from water, which had a GSD of 1.12. These results indicate that aerosols from bacteria suspended in water-only medium have a smaller range of particle sizes than those in other media. Also, these data show that other media produce particle size ranges of similar variability, although their median sizes are different. Saline, 0.80% NaCl, contributed significantly to an increase in particle size in PBS and synthetic saliva compared to that for PB (total salt content, 0.96 versus 0.16%, respectively). Bacteria suspended in serum-only medium had a CMD similar to those suspended in PB. However, their geometric sizes may have been different due to differences in structure and specific gravity. For instance, serum is likely to form a film around the rod-shaped cells, yielding particles with a specific gravity of 1, whereas salt, once dried, may form crystals with a specific gravity of about 2 (KH_2PO_4, 2.3; Na_2HPO_4, 2.1; KCl, 2.0).

Table 2 compares culturable aerosol concentrations and particle sizes at low and high RH. Culturable aerosol concentration and particle size increased as the RH increased from 36 to 68%, and the magnitude of the increase was related to the

TABLE 1. Distribution of particle sizes of culturable *S. marcescens* from different suspending media aerosolized by a nebulizer[a] at 36% RH

Stage	Stage size range (μm)[b]	Suspending medium				
		Water only	PBS	Synthetic saliva	PB	Serum only
1	>7	0.33 ± 0.64[c]	0.36 ± 0.71	0.32 ± 0.10	ND[d]	ND
2	4.7–7	0.25 ± 0.29	0.26 ± 0.04	0.18 ± 0.09	ND	ND
3	3.3–4.7	0.25 ± 0.29	2.10 ± 1.40	1.20 ± 0.11	ND	ND
4	2.1–3.3	0.28 ± 0.54	**16.0 ± 3.00**	**13.0 ± 0.28**	2.00 ± 0.21	0.91 ± 0.19
5	1.1–2.1	3.10 ± 2.30	**61.0 ± 3.40**	**60.0 ± 2.70**	**37.0 ± 4.10**	**29.0 ± 2.30**
6	0.65–1.1	**96.0 ± 2.70**[e]	**21.0 ± 2.80**	**26.0 ± 2.60**	**61.0 ± 4.10**	**70.0 ± 2.30**
Total culturable particles (particles m^{-3})		4,541	4,591	8,495	13,238	27,227
CMD (μm)		0.88	1.50	1.40	1.00	0.95
GSD		1.12	1.67	1.50	1.50	1.58

[a] A six-jet Collison nebulizer (model CN-38; BGI) running at 20 lb/in^2.
[b] Stage size ranges of the six-stage Andersen samplers were determined by the manufacturer (Andersen Samplers, Inc.).
[c] Percentage of recovered cells from each stage of the Andersen sampler at UV off mode (mean ± confidence interval; $\alpha = 0.05$).
[d] ND, not determined.
[e] Data in bold were used for UV off/on comparison and UVGI susceptibility calculation, with >30 CFU on agar plates.

TABLE 2. Change in aerosolized culturable cell concentration and *S. marcescens* particle size with change in RH[a]

Stage	Stage size range[b] (μm)	Suspending medium[d]				
		Water only		PBS		Serum only (high RH)
		Low RH	High RH	Low RH	High RH	
4	2.1–3.3	0	0	20 ± 3.9	23 ± 0.8	4.7 ± 2.3
5	1.1–2.1	2.6 ± 1.7[c]	36 ± 4.1	65 ± 4.9	56 ± 6.3	41 ± 5.1
6	0.65–1.1	97 ± 1.7	67 ± 1.4	15 ± 1.0	13 ± 2.1	51 ± 4.1
Total culturable particles (particles m^{-3})		683	859	5,385	19,498	13,376
CMD (μm)		0.88	0.95	1.60	1.70	1.10
GSD		1.12	1.50	1.56	1.56	1.56

[a] Low RH, 36%; high RH, 68%.
[b] Stage size ranges of the six-stage Andersen samplers were determined by the manufacturer (Andersen Samplers, Inc.).
[c] Percentage of recovered cells from each stage of the Andersen sampler at UV off mode (mean ± confidence interval, $\alpha = 0.05$).
[d] Suspending media were aerosolized with a six-jet Collison nebulizer (model CN-38; BGI) running at 20 lb/in^2.

suspending medium. In water suspension, culturable concentrations increased by 26% at the higher RH, and this increase occurred at particles sizes between 1.1 to 2.1 μm. For particles suspended in PBS, the increase in RH more than tripled the culturable cell concentrations at the lower RH, and the degree of change was greatest for larger particles. These results imply that increased survival at high RH is related to an increase in particle size.

The CMD and GSD were also compared at the low- and high-RH levels. CMD increased 8% for particles suspended in water but increased only 6% for particles in PBS. The GSD of the particle size distributions at high RH were similar for water and PBS (about 1.53).

For the serum-only medium, a direct comparison between the cell concentration at low and high RH was not undertaken because of foaming in the nebulizer reservoir. The CMD and GSD of particles in serum at high RH were calculated (Table 2) and compared with prior data collected at low RH (Table 1). Median size increased by 16% when RH increased from 36 to 68%.

Comparison of UVGI susceptibility of *S. marcescens* aerosolized in different suspending media. At 36% RH, *S. marcescens* suspended in water-only and serum-only medium had the highest Z (2.2 and 1.7 m^2 J^{-1}, respectively) and therefore was most susceptible to UVGI. Cells in PB, saliva, and PBS (Z = 0.72 to 0.92 m^2 J^{-1}) were less susceptible to UVGI damage (Table 3). By comparing UVGI susceptibility of particles at stages 4, 5, and 6 of the six-stage sampler, we found the protective effect of saliva and PBS at low RH to be directly related to the size of the culturable particles, with decreasing UVGI susceptibility as the particle size decreased (Table 3). A linear regression line was determined for the Z of the bacteria in PBS in relation to their particle diameter (Z = 0.36 diameter + 0.12; $R^2 = 0.97$). This relation was not observed in the bacteria suspended in serum-only or PB medium. At the higher RH, UVGI susceptibility decreased, the amount of the decrease depending on the composition of the suspending medium (Table 3). For PBS, the magnitude of protection was not related to particle size, whereas for the serum-only medium the protective effect was highest for the largest particles. In some replicates the protective effect of serum on the large particles was impeccable; negative Z values were recorded, indicating that the number of CFU recovered at the UV-on setting was higher than that at the UV-off setting. Although the difference in CFU between these UV-on and -off conditions was within the variation of CFU among the UV-off samples, these negative Z values contributed to an increase in confidence interval (Table 3, serum-only, high RH).

Particle concentration and UVGI effectiveness. Since measured particle size distributions were the same with and without bacterial cells in the media (except in water-only medium) and with and without UVGI operation, we report aerosolization data only from media containing bacterial cells at the UV-off mode. The size distributions of aerosolized particles

TABLE 3. UVGI susceptibility (Z) of aerosolized culturable *S. marcescens* from different suspending media and RH[a] by a nebulizer[b]

Sampler stage[c]	Suspending media							
	Water only		PBS		Serum only		Synthetic saliva, low RH	PB, low RH
	Low RH	High RH	Low RH	High RH	Low RH	High RH		
4			1.07 ± 0.56	0.12 ± 0.11		−0.17 ± 0.45	1.40 ± 0.02	
5			0.77 ± 0.19	0.10 ± 0.04	1.30 ± 0.32	0.48 ± 0.82	0.75 ± 0.01	0.80 ± 0.17
6	2.20 ± 0.35[d]	0.92 ± 0.16	0.40 ± 0.12	0.14 ± 0.03	1.90 ± 0.51	0.78 ± 0.33	0.81 ± 0.22	1.03 ± 0.17
Total[e]	2.20 ± 0.35a	0.92 ± 0.16b	0.72 ± 0.18b	0.11 ± 0.02c	1.70 ± 0.37a	0.61 ± 0.26b	0.84 ± 0.04b	0.92 ± 0.16b

[a] Low RH, 36%; high RH, 68%.
[b] A six-jet Collison nebulizer (model CN-38; BGI) running at 20 lb/in^2.
[c] Stage size range of the six-stage Andersen samplers (in micrograms) 4, 2.1 to 3.3; 5, 1.1 to 2.1; 6, 0.65 to 1.1 (Andersen Samplers, Inc.).
[d] UV susceptibility, in square meters per joule (mean ± confidence interval, $\alpha = 0.05$), for stages that have >30 CFU at UV off mode.
[e] Z values from the sum of all stages that had >30 CFU. The same letter after a given value indicates that the values are not statistically different (Bonferroni correction for multiple comparisons, $P < 0.05$).

TABLE 4. Total particle count and mean size of different suspending media aerosolized with *S. marcescens* by a nebulizer[a] at 36% RH

Measurement criterion	Reference materials, no cells			Suspending medium, with cells			
	DOP[b]	Cabinet air[c]	Water only	Water only	PBS	Synthetic saliva	Serum only
Total no. of particles[d] ($\times 10^6$ m^{-3})	194	0	0.02	4.60	237	250	301
CMD (μm)	1.25	0	0.81	1.00	1.40	1.60	0.87
GSD	1.29	0	1.11	1.25	1.20	1.20	1.26

[a] A six-jet Collison nebulizer (model CN-38; BGI) running at 20 psi.
[b] DOP, a nonvolatile reference material for measuring the size of the aerosol discharged from the nebulizer.
[c] Background air.
[d] Settings of the API Aerosizer (TSI Inc.). Follow: size range, 0.1 to 10 μm; density, 1 g m^{-3}; run length, 1 min at 2.5 liters min^{-1}.

with and without cells inside were measured for a range of media and compared to a DOP aerosol, all at 36% RH (Table 4). The anomalous presence of particles in aerosols from water without cells was probably due to some cell residues left in the nebulizer from previous uses. The concentration of these residual particles ($0.02 - 10^6$ m^{-3}) was insignificant compared to the number of aerosol particles produced when cells were added ($4.6 - 10^6$ m^{-3}).

The ratio of total to culturable particles was as high as 50,000 times greater (for PBS, 237×10^6/4,591 per m^3). To investigate the potential shadowing effect of the inert particles, *S. marcescens* in a water-only medium in one nebulizer and PBS plus 0.8% extra NaCl without cells in a second nebulizer were operated simultaneously. Under this condition, about 50,000:1 (total:culturable) particles at CMD of 2.1 μm were generated. The UVGI susceptibility of *S. marcescens* under these conditions was then compared to the susceptibility of bacterial particles aerosolized in water-only medium at high RH to cancel out the humidity effect created when two nebulizers are used. The result indicated no difference in UVGI susceptibility ($Z = 0.92 \pm 0.16$ m^2 J^{-1}), and therefore there was no shadowing effect on UVGI efficacy under our experimental conditions. In this experiment, all the particles were measured in terms of their aerodynamic equivalent diameter. However, it is likely that the physical diameter of these particles was different, depending on their specific gravity, shape (dynamic shape factors), and size (slip correction factor for less than 1-μm particles) (6); geometric particle size was not calculated.

Comparing deactivation by humidity and UVGI exposure. Both UVGI exposure and drying at low RH could decrease the number of culturable bacteria. To evaluate the comparative role of UVGI and drying, we compared the effectiveness of these two interventions in deactivating *S. marcescens*. The UVGI doses required to achieve the same reduction in bacterial counts when RH decreased from 68 to 36% with PBS and water-only medium were calculated as follows:

$$\text{Fraction remaining after reducing RH} = \text{counts}_{\text{low RH}}/\text{counts}_{\text{high RH}}$$

$$\text{in water} = 683/859 = 0.795;$$
$$\text{in PBS} = 5385/19498 = 0.276$$

$$\text{UV dose required to achieve equivalent reduction} = \frac{\ln(1/\text{fraction remaining})}{\text{UVGI susceptibility}_{\text{high RH}}}$$

$$\text{in water} = \ln(1/0.795)/0.92 = 0.25 \text{ J m}^{-2}$$
$$\text{in PBS} = \ln(1/0.276)/0.11 = 11.70 \text{ J m}^{-2}$$

UVGI doses were required to achieve the same reduction in culturable particles as that for decreasing RH were 0.25 J m^{-2} for water-only medium and 11.70 J m^{-2} for PBS medium, both under high-RH conditions.

DISCUSSION

Particle size. Determination and prediction of bioaerosol particle sizes from different sources are important, because most health effects induced by airborne biological agents are determined by the success of deposition onto targeted sites. Changes induced in the size of bioaerosols by suspending media are complicated. Variations in size in different media may be related to swelling or shrinking of the cells, adhesion of nonvolatile components of the suspending medium, more or less clumping due to media influence, or two or more of these effects. Direct effects of suspending media in particle size can be understood by the fact that microorganism-containing droplets dry out quickly to a steady state after they become airborne and that the dried nonvolatile residue of the suspending medium remains on the surface of the microorganisms (2). The degree of dryness at steady state depends on the properties and hygroscopicity of the medium and the microorganisms per se and on their response to RH in the environment (6). Therefore, the droplet size, composition of the suspending medium, and RH, all of which control the extent of drying, are critical in determining the ultimate particle size in air. In this study, all three factors were investigated.

In these experiments, a relatively diluted concentration of bacterial cells was used compared to the number of droplets aerosolized, making it likely that only one bacterial cell was embraced in most aerosol particles. The fact that nearly all the airborne bacteria suspended in water-only medium were collected on the impactor stage, retaining the size range of 0.65 to 1.1 μm, supported this hypothesis. According to the size of particles, *S. marcescens* suspended in water-only medium produced aerosols that would deposit primarily in the alveolar region (0.65 to 1.1 μm), while those suspended in PB and serum-only medium were of a size that would likely settle in both terminal bronchi (1.1 to 2.1 μm) and the alveoli. Finally, aerosols in PBS and saliva covered a wider spectrum of particle sizes, some of which could deposit in secondary bronchi (2.1 to 3.3 μm) to alveoli (Andersen Samplers, Inc.) (6).

Given similar droplet size distributions, the GSD for all media except water-only medium were similar (GSD = 1.56). Water suspension produced relatively monodisperse culturable particles, a logical result because water evaporates and leaves no residue around the cells. For other suspending media, because nebulized droplet size distribution was constant, the CMD reflects the influence of different suspending media. Increases in cell size and in cultural recovery with increasing RH have previously been reported and discussed (7, 12, 13). Our data are the first to compare the influence of different suspending media. We demonstrated that the size of particles

from serum-only suspending medium is more responsive to an increase in RH than those from water-only and PBS media.

Suspending medium and UVGI susceptibility at low RH. The influence of suspending medium on UVGI susceptibility has been clearly demonstrated in this study. Thus, outcomes from different laboratories for these types of experiments are likely to vary depending on the suspending medium used. Although serum has been reported as a good protective layer for some airborne microorganisms because of its proteinaceous nature (4, 14), its protective value against UVGI exposure was no greater than that of water at low RH. Salt particles were strongly protective against UVGI deactivation, perhaps because of their UV absorption properties and the formation of a physical barrier (3). Although PB and serum produced culturable particles of similar sizes, PB was more protective than serum. Moreover, although PBS and saliva produced larger particles than PB, total UVGI susceptibility was statistically the same as that for PB. This result suggests that particle size is not the sole factor determining UVGI susceptibility. On the other hand, compared to water, increase in particle size by nebulizing with different suspending media was a good indicator of decreasing UVGI susceptibility.

Humidity, particle size, and UVGI susceptibility. At low RH, cells in PBS exhibited an increase in UVGI susceptibility with increased particle size. Similar results were obtained for cells suspended in synthetic saliva. In serum-only suspension, UVGI susceptibility was unrelated to size at low RH, but at high RH, susceptibility increased as size decreased, a reverse trend to that for PBS and saliva.

These results may be explained by the properties of the media. We suspected that, for media containing salt, the formation of large crystals under dry conditions may lead to gaps in the salt layer, allowing UVGI penetration to the cells. Salt forms cubical crystals, so that the larger the crystals (and hence the particle size), the larger the gaps. However, this phenomenon disappeared at high RH, possibly because the salt dissolves in water, forming a homogenous UVGI-absorbing layer. Although we demonstrated that bacterial aerosols held more water at 68% RH than that at 36%, we did not investigate the state of the salt, crystallized or dissolved, around the bacteria under these RH conditions. For serum suspensions, it has been reported that this protein-rich viscous layer could keep the aerosolized bacteria, i.e., *Klebsiella pneumoniae*, in a hydrated state at RH above 45% (4). It is likely that the role of the serum coating on the particles was to reduce evaporation of water (4). *Serratia* and *Klebsiella* are in the same family, *Enterobacteriaceae* (5); thus, it is reasonable that serum provides a similar protective effect on *S. marcescens*. Our data support this hypothesis. The difference in CMD from low to high RH was 16% for serum-coated particles, compared to 8% for water-only medium and 6% for PBS. Under dry conditions (36%), the serum layer probably dried out and lost its function of water retention. Larger serum particles lower UVGI susceptibility at 68% RH, emphasizing the role of water in protection from UV-induced cell damage. Although different relationships between particle size and UVGI susceptibility were shown in this study at different suspending media and RH conditions, we did not investigate the mechanism involved in these relationships. Particle size may act directly or indirectly with other associated factors in controlling microbial UVGI susceptibility.

Comparison of our survival curve and UVGI susceptibility in the literature. In our study, *S. marcescens* aerosols exposed to UVGI decayed with a Z value of 2.20 $m^2\ J^{-1}$ when suspended in water-only medium. This result supports the one-hit model for UVGI deactivation. Ko and coworkers (7), who worked in our laboratory with the same exposure system, found a similar one-hit model result; however, the Z values reported at low RH (22 to 33%) were about 0.58 $m^2\ J^{-1}$. These investigators used synthetic saliva in their study and selected the fifth stage only for the calculation of UVGI susceptibility. If we compare our data by using the same medium and particle size, the Z value becomes 0.75 $m^2\ J^{-1}$ (Table 3), which is reasonably close to the value found by Ko et al. (7). This example demonstrates how to apply our results to compare UVGI susceptibility from different studies.

We also compared our results with those of Riley and Kaufman (13). These investigators did not describe the suspending medium; however, the fractions of total counts on stages of their cascade impactor were 7, 56, and 33% on the fourth, fifth, and sixth stages, respectively. One can hypothesize that they suspended their bacteria in PBS because of the similarity of their culturable particle size distribution to ours with that suspending medium (Table 1). In their study, the counts on stages 5 and 6 were combined for the calculation of the fraction remaining after UVGI exposure, but UVGI susceptibility was not calculated. Because survival curves were plotted in their study, it is possible to calculate the Z values from the plots. A two-stage relationship, which is different from results of studies both by us and by Ko et al. (7), was found. The survival curves from Riley and Kaufman's data indicated two subpopulations in the samples that were described by the equation

$$N_{UV}/N_0 = F_1 e^{-K_1 D} + F_2 e^{-K_2 D}$$

where N_0 and N_{UV} are the counts before and after UVGI exposure, F_1 and F_2 are the fractions of the subpopulations 1 and 2, K_1 and K_2 are the UVGI susceptibilities of subpopulations 1 and 2, and D is the UVGI dose. In this case, two Z values, 19 and 2.00 $m^2\ J^{-1}$, were calculated from Riley and Kaufman's data at 30% RH; both values are much higher than those determined in our study.

We then investigated the hypothesis that the subpopulation effect shown in the Riley and Kaufman study is due to the aerosolization of a range of particles with various sizes and UVGI susceptibility. For our survival curve, the bacteria were suspended in water-only medium, which produced 96% of culturable particles at size range 0.65 to 1.1 μm. Therefore, we were evaluating the UVGI susceptibility of a group of similar-sized particles within a size range of 0.65 to 1.1 μm. Similarly, the data of Ko et al. were derived from a single stage (fifth stage) representing a small range of particle sizes, and a straight line was expected. However, Riley and Kaufman combined the counts on the fifth and sixth stages. In our study with PBS, particles from different stages had different susceptibilities to UVGI: 1 $m^2\ J^{-1}$ for fourth, 0.8 $m^2\ J^{-1}$ for fifth, and 0.4 $m^2\ J^{-1}$ for sixth stage. These data support the assumption that the Riley and Kaufman samples were likely to contain more than one population of particles that had different UVGI sus-

ceptibilities due to differences in particle size produced by the medium. However, the importance of the change in UVGI susceptibility by particle size in producing this two-stage relationship in their study cannot be known without considering all factors, such as nebulization and aerosolization conditions. In addition to our proposed cause of variation of UVGI susceptibility in a population, the range of particle sizes aerosolized, other alternate and prevailing viewpoints on this issue were discussed by Kowalski et al. (9).

Particle shading on UVGI efficacy. In our study, the presence of aerosols did not cause a reduction in UVGI efficacy. We calculated the size of aerosolized PBS particles, assuming that they (i) are fully dry, (ii) have a cubic shape, (iii) have a density of 2.17 g ml^{-1}, (iv) form droplets of 1.25 μm in diameter, and (v) have a salt concentration of 0.96% in the liquid medium. In that case, each PBS particle contains 9.74×10^{-15} g of salt, which is equivalent to a cube with a length of 0.17 μm. We also calculated the volume of air occupied by one aerosol particle as 5 mm^3, assuming 2×10^8 particles per m^3 of air. These calculations supported the conclusion that the aerosol particles were widely spaced and would shade only a very small portion of the cell.

Implications of experimental results. This study compared several laboratory-used suspending media with respect to their effects on the sizes of airborne aerosolized bacteria and their susceptibilities to UVGI. This information is valuable for data comparison and deciding on an appropriate medium to use for various applications. Moreover, we proposed a new mechanism by which laboratory-determined UVGI susceptibility may be changed due to the variation in particle size in different suspending media. We recommend using a suspending medium that is similar to the source of microorganisms for laboratory UVGI studies, for instance, using synthetic saliva to simulate aerosols discharged from human respiratory systems.

ACKNOWLEDGMENTS

K.M.L. is grateful to the Croucher Foundation, Hong Kong, for the award of a postdoctoral research fellowship. We thank the Tuberculosis UV Shelter Study (TUSS) for helping to support this work.

We thank Ed Nardell, Christine Rogers, and Steve Rudnick for reviewing the manuscript and Kevin Banahan for technical assistance with the experimental chamber.

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